93. (New) The enzyme mixture of claim 68, wherein when said mutant DNA polymerase comprising a mutation in its partitioning domain or polymerase domain is a mutant PGB-D (Deep Vent) DNA polymerase, said mutant PGB-D (Deep Vent) DNA polymerase contains a mutation at an amino acid position selected from the group consisting of: D405, T542, D543, K593, Y385, G387, and G388.

94. (New) The enzyme mixture of claim 93, wherein said mutant PGB-D (Deep Vent) DNA polymerase contains a mutation of G387P.

REMARKS

Pursuant to MPEP 706.07(h), on filing an RCE, Applicant may direct that the previously filed, but un-entered After-final amendment not be entered, but that, instead, the present submission be entered for consideration by the Examiner. Accordingly, Applicant requests that the previously filed After-final amendment (filed on September 2, 2004) not be entered into the file and request entry of the instant amendments and remarks.

Claims 64-94 are currently pending in the application. Claims 65, 67-70, 71-74, 76 and 86 are amended. Claim 69 is cancelled. The amendments are supported in the specification as specifically discussed below. Claims 88-94 are added. The added claims 88-94 are supported throughout the specification, e.g., on pages 27-29 (Tables 2A and 2B), and claim 88 is also supported in previous claim 74 before its amendment. No new matter is added.

Amendments to the Specification

Table 2B on page 28 is amended to correct clerical errors regarding the position of specific mutations for the Vent and Deep Vent DNA polymerases. The amended positions are supported in Table 2B as originally filed. No new matter is added. Applicants respectfully request the entry of the amendments.

In a telephone interview of June 21, 2004, Applicants and the Examiner has agreed to incorporate the sequences for JDF-3 DNA polymerase into the present specification, although Applicants believe the specification as originally filed (i.e., without the incorporation) already satisfies the enablement requirement.

Such incorporation is supported under MPEP 608.01(p). Applicants respectfully request the entry of the incorporation.

Claim Objection

Claim 72 is objected to because of the recitation of "mutant KDO DNA polymerase." Applicants have corrected the clerical error to recite "mutant KOD DNA polymerase." Applicants respectfully request the withdrawn of the claim objection on claim 72.

Claim Rejections under 35 U.S.C. §112, Second Paragraph

Claims 69-74 are rejected under 35 U.S.C. §112, second paragraph. The Office Action states that claims 69-74 are indefinite because it is not clear the extent to which the genus if further limited. The Office Action states:

> "The problem is that claim 69 makes no limitation that the claimed mutant polymerase must be a mutant Pfu DNA polymerase thus in effect claim 69 is drawn to the enzyme mixture of claim 67, wherein said mutant DNA polymerase comrpising a mutation in its partitioning domain or polymerase domain is a mutant Pfu DNA polymerase, KOD DNA polymerase, or JDF-3 DNA polymerase, wherein said mutant DNA mutant Pfu DNA polymerase contains a mutation at an amino acid position selected from the group consisting of at D405, Y410, T542,. D543, K593, Y595, Y385, G387 or G388. Thus claim 69 still encompasses the specific amino acid mutants of Pfu as well as partitioning domain or polymerase domain mutants of KOD DNA polymerase, or JDF-3 DNA polymerase."

Applicants respectfully disagree. Applicants submit that the claims as written were clear as to the extent that they further limit claim 68. However, for the sole purpose of expediting prosecution, Applicants have amended claims 69, 71-74. Claims 69, 71-74 are amended. The amendment finds support on pages 27-29 (Tables 2A and 2B).

Applicants submit that the above claim amendments obviates the 35 U.S.C. 112, second paragraph rejections on claims 69-74. Applicants respectfully request the withdrawn of the indefiniteness rejections on claims 69-74.

Claim Rejections under 35 U.S.C. §112, First Paragraph

Claims 65, 66, 68-69, 73-74, 76-81 and 83-84 are rejected under 35 U.S.C. §112, first paragraph. The Office Action states that these claims are not enabling and that a deposit of the referred bacterial DNA polymerases is required.

Applicants respectfully disagree. In an interview with Applicants' representatives on June 21, 2004 (see Statement of Substance submitted herewith), Examiner Hutson clarified that the enablement rejection was issued in particular because of the recitation of the JDF-3 DNA polymerase. The Examiner stated a deposit for JDF-3, not for any other DNA polymerases recited in the claims, was required because JDF-3 DNA polymerase was not deemed readily accessible to the public.

Applicants respectfully disagree. Applicants submit that all DNA polymerases recited which can be used as the first enzyme of the present invention, including the JDF-3 DNA polymerase, are known in the art and they are readily accessible to the public. No deposit for any of the DNA polymerases is required.

First, Applicants submit that all recited DNA polymerases are well known in the art. As stated in the previous response filed December 18, 2003, the specification provides sequence accession number for each of the claimed DNA polymerase (e.g., pages 14-19). The specification further provides at least one publication reference for each of the DNA polymerases recited in the rejected claims (e.g., on page 12).

Second, Applicants submit that the recited DNA polymerases are readily accessible to the public. It is routine for one skilled in the art of molecular biology to express a protein based on its known nucleotide or amino acid sequence. The instant specification specifically teaches the expression and purification of a DNA polymerase (mutant or wild-type) using a polynucleotide

encoding the DNA polymerase (e.g., pages 33-34, Example 2). No undue experimentation is required for such routine expression and purification of any of the DNA polymerases recited.

In addition to the teachings of the specification, many of the DNA polymerases were commercially available and their availability was known to one skilled in the art. For example, page 16 of the present specification provides the availability of some DNA polymerases from various commercial sources. Applicants herein further provide more detailed information on the commercial availability of the DNA polymerases:

DNA polymerases	**Vendor**	**Catalog #**
Taq DNA polymerase	Stratagene, La Jolla, CA	600131, 600132, 600139
	Promega, Madison, WI	M1661, M1665, M1668, M1861, M1865
Tth DNA polymerase	Promega, Madison, WI	M2101, M2105
Tli (Vent) DNA polymerase	New England Biolabs, Beverly, MA	254S
	Promega, Madison, WI	M7101
Tgo DNA polymerase	Roche Applied Science, Indianapolis, IN	3186172
Pfu DNA polymerase	Stratagene, La Jolla, CA	600135, 600136, 600140,
	Promega, Madison, WI	M7741, M7745
KOD DNA polymerase	Novagen, San Diego, CA	71085-3

PGB-D (Deep Vent) DNA polymerase	New England Biolabs, Beverly, MA	258S, 258L
Pwo DNA polymerase	Boehringer Mannheim, Indianapolis, IN	1644947

Third, Examiner Hutson clarified during the interview that no deposit was required for any other DNA polymerases, but the claims were rejected for their recitation of "JDF-3 DNA polymerase." The Examiner felt that the JDF-3 DNA polymerase was not readily accessible to the public.

With respect to **JDF-3 DNA polymerase** particularly, Applicants submit that JDF-3 DNA polymerase was not only known in the art, but also readily accessible to the public as of the instant patent application filing date. For example, JDF-3 DNA polymerase is described on page 12 (lines 19-20 During our June 21, 2004 telephone interview, Examiner Hutson agreed with Applicants' that JDF-3 polypeptide sequence was available, but maintained that the phrase "JDF-3 DNA polymerase," as used in the claims, even in view of the teachings in the specification, did not specifically disclose the particular JDF-3 sequence which would enable the making and using of the JDF-3 DNA polymerase in the claimed invention. Although Applicants believe the above teachings satisfy the enablement requirement for JDF-3 DNA polymerase, for the sole purpose of expediting the prosecution, Applicants thereby further incorporate JDF-3 amino acid sequence (SEQ ID NO:2 of WO 01/32887) and its corresponding DNA sequence (SEQ ID NO:1 of WO 01/32887) into the present specification. The incorporation of the sequences is permitted under MPEP 608.01(p). Examiner Hutson agreed that such incorporation would obviates the enablement rejections on claims 65-66, 68-69, 73-74, 76-81 and 83-84 because of the recitation of JDF-3 DNA polymerase. During the interview of June 21, 2004, Applicants' representative, by mistake, referred to US Patent No. 5,602,011 recited on page 16 of the specification as the patent that contains the JDf-3 sequences. Applicants wish to correct the mistake and state that WO 01/32887 recited on page 12 of the present specification contains the correct JDF-3 polypeptide and nucleotide sequences.

In view of the above, Applicants submit that all DNA polymerases recited in claims 65-66, 68-69, 73-74, 76-81 and 83-84 are known in the art and are readily accessible to the public and/or they can be obtained without undue experimentation. One skilled in the art, therefore, will know how to make and use the present invention as claimed based on the teaching of the present specification. Applicants, therefore, respectfully request the lack of enablement rejections under112, first paragraph over claims 65-66, 68-69, 73-74, 76-81 and 83-84 be withdrawn.

Claim Rejections under 35 U.S.C. §103(a)

Claims 64-69, 75, 82-83 and 85-87 are rejected under 35 U.S.C. §103(a). The Office Action states that the claims are obvious over Barnes et al. (U.S. Patent No. 5,436,149) and Komori et al.

Applicants respectfully disagree based on the same reasoning presented in the previous response filed December 18, 2003.

Claims 64-66, 75 and 85

With respect to claims 64-66, 75 and 85, Applicants submit that these claims are drawn to an enzyme mixture containing a first enzyme and a second enzyme, *wherein said **first enzyme is an Archaeal DNA polymerase**, said second enzyme is a mutant **Archaeal DNA polymerase with a 3'-5' exonuclease activity** and a reduced DNA polymerization activity.* Therefore, the first enzyme is limited to an Archaeal DNA polymerase and the second enzyme is limited to an enzyme with a 3'-5' exonuclease activity (exo^+). As known in the art, all archaeal DNA polymerases contain 3'-5' exonuclease activity (e.g., See Exhibit A, page 327 and Figure 4, relevant text highlighted). Therefore, both the first and the second enzymes of the enzyme mixture as claimed in claims 64-66, 75 and 85 contain the 3'-5' exonuclease activity. That is, the enzyme mixture of the invention as claimed in 64-66, 75 and 85 comprises **two exo^+ enzymes**.

First, neither Barnes et al. nor Komori et al. teaches or suggest the present invention as claimed in claims 64-66, 75 and 85. Barnes et al. describes a formulation with a **majority DNA**

polymerase component **lacking 3'-5' exonuclease activity** (**exo$^-$**, e.g., Taq DNA polymerase) and a **minority DNA polymerase** component **exhibiting 3'-5' exonuclease activity** (**exo$^+$**, e.g., wild type Pfu DNA polymerase). Therefore, the formulation in Barnes et al. comprises an **exo$^-$** enzyme **and** an **exo$^+$** enzyme. In contrast to the teachings in Barnes et al., claims 64-66, 75 and 85 of the present invention claim an enzyme mixture comprising a first enzyme and a second enzyme, wherein the first enzyme is an Archaeal DNA polymerase (**one exo$^+$**) and said second enzyme is a mutant Archaeal DNA polymerase comprising a 3'-5' exonuclease activity and a reduced DNA polymerization activity (**another exo$^+$**). Barnes et al. does not teach or suggest an enzyme mixture of two exo$^+$ enzymes. Komori et al. does not teach or suggest such an enzyme mixture either.

Second, there is no suggestion or motivation to combine the prior art references. In order to establish a prima facie case of obviousness, there must be some reason, suggestion, or motivation from the prior art as a whole that indicates that the person of ordinary skill would have combined or modified the references. The Federal Circuit has stated:

> "[O]bviousness cannot be established by combining the teachings of the prior art to produce the claimed invention, absent some teaching, suggestion or incentive supporting the combination."[1]

As stated above, Barnes et al. teaches a formulation of one exo$^-$ DNA polymerase and another exo$^+$ DNA polymerase. The formulation is provided based on the theory that the use of a DNA polymerase exhibiting *3'-exonuclease activity* (exo$^+$) can overcome the problem of a DNA polymerase lacking *3'-exonuclease activity* (exo$^-$), e.g., Taq DNA polymerase. Barnes et al. provides:

> "As speculated in Barnes (1992; supra), Thermus aquaticus DNA polymerase and its variants are slow to extend a mismatched base pain (which they cannot remove since they lack any 3'-exonuclease. A couple of companies (New England Biolabs and Stratagene) have introduced thermostable enzymes which exhibit a 3'-(editing) exonuclease which should, one would think, allow the removal of

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[1] *In re Geiger*, 815 F.2d 686, 688, 2 U.S.P.Q.2d 1279, 1278 (Fed. Cir. 1987)

> mismatched bases to result in both efficient extension and more accurately copied products. In practice, these two enzymes (Vent and Pfu DNA polymerase) are unreliable and much less efficient than expected....
>
> I have discovered that the expected beneficial effects of a 3'-exonuclease can be obtained with an unexpectedly minute presence of an Archaebacterial DNA polymerase, whilst efficient extension is being catalyzed by a large amount of (3'-exonuclease-free) KlenTaq-278 or AT." (Columns 16-17).

As one can see, Barnes specifically teaches a combination of *an exo$^-$ and an exo$^+$ DNA polymerases* so that *the presence of the exo+ DNA polymerase increases the amplification efficiency of the exo$^-$ DNA polymerase in the mixture.* Barnes et al. do not teach or suggest a enzyme mixture of an exo$^+$ DNA polymerase with another exo$^+$ DNA polymerase with reduced polymerization activity. In fact, if the first enzyme is an Archaeal DNA polymerase which already possesses the 3'-5' exonuclease activity, as claimed in claims 64-66, 75 and 85 of the present invention, there would be no motivation for one skilled in the art to mix the first enzyme with another exo$^+$ enzyme based on the teachings of Barnes et al. Therefore, it would not be obvious for one skilled in the art, in the absence of the present teaching, to make an enzyme mixture comprising a first and a second enzyme, where both enzymes contain 3'-5' exonuclease activity as claimed in claims 64-66, 75 and 85 of the present invention.

Komori et al. studies the structure-function relationship of Pfu DNA polymerase, i.e., what mutations affect or abolish the DNA polymerase and exonuclease activities of Pfu. Komori et al. does not teach or suggests that these two mutants can be used with another DNA polymerase, let alone be used with another exo$^+$ DNA polymerase (Archaeal DNA polymerase) as claimed in the present invention.

Therefore, there is no teaching or suggestion to combine or modify Barnes et al. and Komori et al. to reach to the present invention, that is, an enzyme mixture comprising a first enzyme and a second enzyme, wherein the first enzyme is an Archaeal DNA polymerase and the second enzyme is a mutant Archaeal DNA polymerase comprising 3'-5' exonuclease activity and a reduced DNA polymerization activity. Because the prior art references fail to provide any suggestion or incentive to combine or modify the references, the Office Action fails to establish a prima facie case of obviousness.

Third, even when the prior art references are combined, they do not result in the present invention as claimed in claims 64-66, 75 and 85. The combination of Barnes et al. and Komori et al. still does not teach or suggest an enzyme mixture comprising a first enzyme and a second enzyme, wherein the first enzyme is an Archaeal DNA polymerase and the second enzyme is a mutant Archaeal DNA polymerase comprising 3'-5' exonuclease activity and a reduced DNA polymerization activity, that is, both enzymes are exo^+ enzymes.

In view of the above, Applicants submit that neither Barnes et al, or Komori et al., alone or in combination, teach or suggest the invention as claimed in claims 64-66, 75 and 85 of the present invention, i.e., to make an enzyme mixture comprising a first enzyme and a second enzyme, where the *first enzyme is an Archaeal DNA polymerase, said second enzyme is a mutant Archaeal DNA polymerase with a 3'-5' exonuclease activity and a reduced DNA polymerization activity.*

Claims 67-69, 82-83, 86-87

With respect to other claims rejected under 103(a), namely, claims 67-69, 82-83, 86-87, Applicants submit that claims as previously presented are not obvious over Barnes et al. and Komori et al. However, for the sole purpose of expediting the present prosecution, Applicants have amended the claims. Applicants preserve the right of pursuing the subject matters as previously presented in claims 67-69, 82-83, 86-87 (i.e., D405 mutants) in a subsequent continuation application.

First, neither Barnes et al. nor Komori et al. teaches or suggests the present invention as recited in claims 67-69, 82-83, 86-87. Barnes et al. describes a formulation with a majority DNA polymerase component lacking 3'-5' exonuclease activity (e.g., Taq DNA polymerase) and a minority DNA polymerase component exhibiting 3'-5' exonuclease activity (e.g., wild type Pfu DNA polymerase). Komori et al. describes two specific D405 Pfu mutants (i.e., D405A and D405E) that have reduced polymerase activity. Neither Barnes et al. nor Komori et al. teaches or suggests the claimed invention, that is, an enzyme mixture a first enzyme and a second enzyme, wherein said first enzyme is a DNA polymerase, said second enzyme is a mutant Archaeal DNA polymerase comprising a 3'-5' exonuclease activity and a reduced DNA polymerization activity,

wherein when said mutant Archaeal DNA polymerase is a mutant Pfu DNA polymerase, the mutant Pfu DNA polymerase contains a mutation at an amino acid position selected from the group consisting of Y410, T542, D543, K593, Y595, Y385, G387, and G388.

Second, there is no suggestion or motivation to combine the prior art references. In order to establish a prima facie case of obviousness, there must be some reason, suggestion, or motivation from the prior art as a whole that indicates that the person of ordinary skill would have combined or modified the references. The Federal Circuit has stated:

> "[O]bviousness cannot be established by combining the teachings of the prior art to produce the claimed invention, absent some teaching, suggestion or incentive supporting the combination."[2]

As stated above, Barnes et al. describes a formulation with a majority DNA polymerase component lacking 3'-5' exonuclease activity (e.g., Taq DNA polymerase) and a minority DNA polymerase component exhibiting 3'-5' exonuclease activity (e.g., **wild type Pfu DNA polymerase**). Barnes et al. does not teach or suggest a mutant DNA polymerase should be used as the minority DNA polymerase component in the formulation, let alone a mutant Archaeal DNA polymerase comprising a 3'-5' exonuclease activity and a reduced DNA polymerization activity, wherein when said mutant Archaeal DNA polymerase comprising a 3'-5' exonuclease activity and a reduced DNA polymerization activity, the mutant polymerase contains a mutation at an amino acid position selected from the group consisting of Y410, T542, D543, K593, Y595, Y385, G387, and G388.

Komori et al. studies the structure-function relationship of Pfu DNA polymerase, i.e., what mutations affect or abolish the DNA polymerase and exonuclease activities of Pfu:

> "To expand our knowledge of the structure-function relationships fo the family B DNA polymerases, and especially to understand the structural relationship between the DNA polymerizing and 3'-5' exonucleolytic activities in the polymerase protein, we prepared several mutant proteins of Pol BI from *Pfuriosus* by a

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[2] *In re Geiger*, 815 F.2d 686, 688, 2 U.S.P.Q.2d 1279, 1278 (Fed. Cir. 1987)

unidirectional deletion strategy and site-specific mutagenesis, and analyzed their activities." (Page 41, the right column).

"In conclusion, our mutational analysis further supports the idea that the polymerase and exonuclease domains in the family B DNA polymerases are functionally interdependent. More detailed analyses will be necessary to understand the molecular mechanism of the functional interaction between the two activities in the DNA polymerases." (Page 47, last paragraph).

Komori et al. describes two specific **D405** Pfu mutants (i.e., D405A and D405E) that have reduced polymerase activity. Komori et al. does not teach or suggests that these two mutants can be used with another DNA polymerase in the way claimed in the present invention. **In fact, Komori et al. does not teach that these two Pfu mutants can have any utilities at all.** Even without the present amendments for claims 67-69, 82-83 and 86-87, that is, even if the claims still recite the D405 mutation, one skilled in the art, based on the teachings of Komori et al. and absent of the teachings of Applicants' present invention, would likely avoid mutating the D405 residue of Pfu DNA polymerase to preserve its DNA polymerase and exonuclease activity.

Therefore, nothing in the two references teaches or suggests to combine or modify the references to reach to the present invention, that is, an enzyme mixture comprising a first enzyme and a second enzyme, wherein said first enzyme is a DNA polymerase, said second enzyme is a mutant Archaeal DNA polymerase comprising a 3'-5' exonuclease activity and a reduced DNA polymerization activity, wherein when said mutant Archaeal DNA polymerase comprising a 3'-5' exonuclease activity and a reduced DNA polymerization activity is a mutant Pfu DNA polymerase, the mutant Pfu DNA polymerase contains a mutation at an amino acid position selected from the group consisting of Y410, T542, D543, K593, Y595, Y385, G387, and G388. Because the prior art references fail to provide any suggestion or incentive to combine or modify the references, the Office Action fails to establish a prima facie case of obviousness.

Third, even when the prior art references are combined, they do not result in the present invention as claimed. The amended 67-69, 82-83, 86-87, are drawn to an enzyme mixture comprising a first enzyme and a second enzyme, wherein said first enzyme is a DNA polymerase, said second enzyme is a mutant Archaeal DNA polymerase comprising a 3'-5' exonuclease activity and a reduced DNA polymerization activity, wherein when said mutant

Archaeal DNA polymerase comprising a 3'-5' exonuclease activity and a reduced DNA polymerization activity is a mutant Pfu DNA polymerase, the mutant Pfu DNA polymerase contains a mutation at an amino acid position selected from the group consisting of Y410, T542, D543, K593, Y595, Y385, G387, and G388. The combination of Barnes et al. and Komori et al. still does not teach or suggest an enzyme mixture comprising a first enzyme and a mutant Archaeal DNA polymerase comprising a 3'-5' exonuclease activity and a reduced DNA polymerization activity, wherein when said mutant Archaeal DNA polymerase comprising a 3'-5' exonuclease activity and a reduced DNA polymerization activity is a mutant Pfu DNA polymerase, the mutant Pfu DNA polymerase contains a mutation at an amino acid position selected from the group consisting of Y410, T542, D543, K593, Y595, Y385, G387, and G388.

In view of all of the above, Applicants submit that claims 64-69, 75, 82-83, and 85-87 are not obvious over Barnes et al. and Komori et al. Examiner Hutson agreed during the June 21 interview that such amendment would obviate the 103 rejections over claims 64-69, 75, 82-83, and 85-87.

In view of all of the above, Applicants submit that none of the pending claims are obvious over Barnes et al. and Komori et al., Applicants respectively request the 103(a) rejections over these claims be withdrawn.

Obviousness-type Double Patenting

Claims 64-87 are provisionally rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-3, 6, 9-14, 18, 20-22 and 36-51 of copending Application No. 10/035,091. The Examiner states that although the conflicting claims are not identical, they are not patentably distinct from each other.

While not necessarily acquiescing to the rejection, Applicants submit that they will submit a terminal disclaimer to disclaim any portion of a patent issuing from the present application which would extend beyond the term of a patent issuing from the 10/035,091 application, upon notification of allowable claims in the present application.

CONCLUSION

Applicants submit that in view of the foregoing amendments and remarks, all issues relevant to patentability raised in the Office Action have been addressed. Applicants respectfully request the withdrawal of rejections over the pending claims.

Applicant submits that all claims, i.e., claims 64-94, are allowable as written and respectfully request early favorable action by the Examiner. If the Examiner believes that a telephone conversation with Applicant's attorney/agent would expedite prosecution of this application, the Examiner is cordially invited to call the undersigned attorney/agent of record.

Respectfully submitted,

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